

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions and listings of claims in the application:

1. (Currently amended) A method of detecting an analyte of interest in a sample, comprising:

(i) contacting in the presence of a first polyanion (a) the sample containing the analyte[[,] and (b) one or more affinity molecule/charged carrier molecule conjugates, ~~and (c) a first polyanion~~ to form a complex of the analyte and the one or more conjugates, wherein each affinity molecule has an affinity against the analyte, each charged carrier molecule has a net negative charge, and the charged carrier molecule causes a change in a separation property of the analyte by binding to the analyte through the affinity molecule to form a complex of the analyte and the affinity molecule/charged carrier molecule conjugate;

~~(ii) providing a microfluidic device having a separation channel filled with a separation media and a second polyanion added to the separation media, the separation channel having at least one microscale dimension of between about 0.1 and 500 microns;~~

(ii) concentrating the complex in the presence of a second polyanion using a concentration channel in a microfluidic device, the concentration channel having at least one microscale dimension of between about 0.1 and about 500 microns;

~~(iii) electrophoretically separating the complex and any unbound conjugate using the filled separation channel; and~~

(iii) electrophoretically separating in the presence of a third polyanion the complex from any unbound conjugate using a separation channel in a microfluidic device, the separation

channel having at least one microscale dimension of between about 0.1 and about 500 microns;

and

(iv) detecting the complex to identify the presence of the analyte or to determine an amount of the analyte in the sample, wherein the first, ~~and~~ second, and third polyanions reduce interference with separating the complex.

2. (Canceled)

3. (Currently amended) The method of claim 1, wherein the first, second, and third polyanions are independently selected from one or more of polysaccharides, polynucleotides, polypeptides, synthetic macromolecular compounds, or ceramics; or a complex thereof.

4. (Currently amended) The method of claim 1, wherein the first, second, and third polyanions are independently selected from one or more of poly-dIdC, heparin sulfate, dextran sulfate, polytungstic acid, polyanethole sulfonic acid, polyvinyl sulfate, polyacrylate, chondroitin sulfate, plasmid DNA, calf thymus DNA, salmon sperm DNA, DNA coupled to cellulose, glass particles, colloidal glass, or glass milk, or a complex thereof.

5-7 (Canceled)

8. (Currently amended) The method of claim 1, wherein the first, ~~and/or~~ second and/or third polyanion comprises heparin sulfate.

9. (Original) The method of claim 1, wherein at least one of the one or more affinity molecules is labeled with a detectable marker.

10. (Previously presented) The method of claim 1, wherein the contacting step further comprises contacting the sample with one or more non-conjugated affinity molecules, wherein each non-conjugated affinity molecule has an affinity against the analyte, to form a

complex of the analyte, the at least one conjugate, and the at least one non-conjugated affinity molecule.

11. (Previously presented) The method of claim 1 or 10, wherein the affinity molecule is one which binds to the analyte by an interaction selected from a protein-protein interaction, a protein-chemical interaction or a chemical-chemical interaction.

12. (Previously presented) The method of claim 1 or 10, wherein the affinity molecule is one which binds to the analyte by an interaction selected from an antigen-antibody interaction, a sugar chain-lectin interaction, an enzyme-inhibitor interaction, a protein-peptide chain interaction, a chromosome or nucleotide chain-nucleotide chain interaction, a nucleotide-ligand interaction or a receptor-ligand interaction.

13. (Previously presented) The method of claim 1 or 10, wherein the affinity molecule is selected from one or more of an antibody, an Fab, F(ab')₂ or Fab' fragment of an antibody, an antibody variable region, a lectin, avidin, a receptor, an affinity peptide, an aptamer, or a DNA binding protein.

14. (Previously presented) The method of claim 1, wherein the charged carrier molecule is an anionic molecule.

15. (Canceled)

16. (Previously presented) The method of claim 14, wherein the charged carrier molecule is an anionic molecule selected from a nucleotide chain or a sulfonated polypeptide.

17. (Previously presented) The method of claim 1, wherein the charged carrier molecule comprises DNA, RNA, an anionic polymer, or a sulfonated polypeptide

18. (Original) The method of claim 17, wherein the charged carrier molecule comprises DNA comprising one or more synthetic sequences.

19. (Original) The method of claim 18, wherein the one or more synthetic sequences comprise one or more nucleotide analogs comprising a linker group or a linker reactive group.

20. (Previously presented) The method of claim 19, wherein the linker group or linker reactive group is selected from an amino group, a thiol, a carboxyl group, an imidazol group, or a succinimide group.

21. (Original) The method of claim 20, further comprising covalently bonding a detectable marker to the linker group or linker reactive group.

22. (Previously presented) The method of claim 1, wherein at least one of the one or more affinity molecules is labeled with a detectable marker.

23. (Previously presented) The method of claim 10, wherein at least one conjugate or at least one non-conjugated affinity molecule is labeled with a detectable marker.

24. (Previously presented) The method of claim 10, wherein at least one conjugate is labeled by a detectable marker.

25. (Previously presented) The method of claim 10, wherein the charged carrier molecule in at least one conjugate is labeled by a detectable marker.

26. (Previously presented) The method of claim 10, wherein the affinity molecule in at least one conjugate is labeled by a detectable marker.

27. (Previously presented) The method of claim 9, 21, 22, 23, 24, 25 or 26, wherein the detectable marker is selected from one or more of a fluorescent dye, a luminescent dye, a phosphorescent dye, a fluorescent protein, a luminescent protein or particle, a radioactive

tracer, a chemiluminescent compound, a redox mediator, an electrogenic compound, an enzyme, a colloidal gold particle, or a silver particle.

28. (Canceled)

29. (Previously presented) The method of claim 1, wherein the separation media comprises a size exclusion resin, a polyacrylamide gel, polyethylene glycol (PEG), polyethyleneoxide (PEO), a co-polymer of sucrose and epichlorohydrin, polyvinylpyrrolidone (PVP), hydroxyethylcellulose (HEC), poly-N,N-dimethylacrylamide (PDMA), or an agarose gel.

30. (Canceled)

31. (Currently amended) The method of claim 1, wherein the ~~second~~ third polyanion is present in the separation media at a concentration of between about 0.01 to 5%.

32. (Currently amended) The method of claim 1, wherein the ~~second~~ third polyanion is present in the separation media at a concentration of between about 0.05 to 2%.

33-34. (Canceled)

35. (Original) The method of claim 1, wherein the separation channel has at least one cross-sectional microscale dimension of between about 0.1 and 200 microns.

36. (Canceled)

37. (Currently amended) The method of claim 1, wherein:
at least one of the one or more conjugates is labeled by a detectable marker;
step (iii) comprises electrophoretically separating the complex from the at least one conjugate labeled by the detectable marker that is not involved in forming the complex ~~using the filled separation channel of the microfluidic device~~; and
step (iv) comprises:

(a) measuring an amount of the separated complex or detecting a presence of the separated complex; and

(b) determining an amount of the analyte in the sample on the basis of the measured amount or identifying a presence of the analyte in the sample on the basis of the detected presence.

38. (Currently amended) The method of claim 10, wherein:

either at least one of the non-conjugated affinity molecules or at least one of the conjugates is labeled by a detectable marker;

step (iii) comprises electrophoretically separating the complex from any free non-conjugated affinity molecule labeled by the detectable marker or any free conjugate labeled by the detectable marker ~~using the filled separation channel of the microfluidic device~~; and

step (iv) comprises:

(a) measuring an amount of the separated complex or detecting a presence of the separated complex; and

(b) determining an amount of the analyte in the sample on the basis of the measured amount or identifying a presence of the analyte in the sample on the basis of the detected presence.

39. (Currently amended) A method for determining an analyte in a sample, the method comprising:

(i) contacting in the presence of a first polyanion (a) the sample containing the analyte, (b) either a labeled analyte formed by labeling analyte extrinsic to the sample with a detectable marker or a labeled analogue of the analyte formed by labeling an analogue with a detectable marker, and (c) one or more affinity molecule/charged carrier molecule conjugates, ~~and (d) a first~~

~~polyanion~~, thereby forming a first complex of the analyte in the sample and the one or more conjugates and a second complex of either the labeled analyte and the one or more conjugates or the labeled analogue and the one or more conjugates; wherein the affinity molecule in each conjugate has an affinity against the analyte in the sample and the labeled analyte, or an affinity against the analyte in the sample and the labeled analogue, and wherein each charged carrier molecule has a net negative charge, and the charged carrier molecule causes a change in a separation property of the analyte or the analogue by binding to the analyte or the analogue through the affinity molecule to form a complex of the analyte or the analogue, with the affinity molecule/charged carrier molecule conjugate;

~~(ii) providing a microfluidic device having a separation channel filled with a separation media and a second polyanion added to the separation media;~~

(ii) concentrating the second complex in the presence of a second polyanion using a concentration channel in a microfluidic device, the concentration channel having at least one microscale dimension of between about 0.1 and about 500 microns;

(iii) electrophoretically separating in the presence of a third polyanion the second complex from any free labeled analyte or free labeled analogue using the filled separation channel using a separation channel in a microfluidic device, the separation channel having at least one microscale dimension of between about 0.1 and about 500 microns;

(iv) measuring an amount of the separated second complex or an amount of the separated free labeled analyte or the separated free labeled analogue; and

(v) determining an amount of the analyte in the sample on the basis of the measured amount; wherein the first, ~~and second,~~ and third polyanions reduce interference with the determination.

40. (Canceled)

41. (Currently amended) The method of claim 39, wherein:

step (i) comprises contacting in the presence of the first polyanion (a) the sample containing the analyte, (b) either the labeled analyte or the labeled analogue, (c) the one or more conjugates, and (d) one or more non-conjugated affinity molecules, ~~and (e) a first polyanion~~, wherein each of the conjugated and non-conjugated affinity molecules have an affinity against the analyte in the sample and the labeled analyte or the analyte in the sample and the labeled analogue, thereby forming a first complex of the analyte in the sample, the non-conjugated affinity molecule, and the conjugate, and a second complex of either the labeled analyte, the non-conjugated affinity molecule, and the conjugate, or the labeled analogue, the non-conjugated affinity molecule, and the conjugate;

step (iii) comprises electrophoretically separating the second complex from any free labeled analyte or free labeled analogue ~~using the filled separation channel of the microfluidic device~~;

step (iv) comprises measuring an amount of the separated second complex or an amount of the separated free labeled analyte or the separated free labeled analogue; and

step (v) comprises determining an amount of the analyte in the sample on the basis of the measured amount.

42. (Currently amended) A method for determining an analyte in a sample, the method comprising:

(i) contacting in the presence of a first polyanion (a) the sample containing the analyte, (b) either a charged carrier molecule-bound analyte formed by binding analyte extrinsic to the sample to a charged carrier molecule or a charged carrier molecule-bound analogue formed by

binding an analogue of the analyte to a charged carrier molecule, and (c) an affinity molecule labeled by a detectable marker, ~~and (d) a first polyanion~~, thereby forming a first complex of either the charged carrier molecule-bound analyte and the labeled affinity molecule or the charged carrier molecule-bound analogue and the labeled affinity molecule and a second complex of the analyte in the sample and the labeled affinity molecule, wherein the affinity molecule has an affinity against the analyte in the sample and the charged carrier molecule-bound analyte or the analyte in the sample and the charged carrier molecule-bound analogue, the charged carrier molecule has a net negative charge, and the charged carrier molecule has a property capable of causing a change in a separation property of the first complex;

~~(ii) providing a microfluidic device having a separation channel filled with a separation media and a second polyanion added to the separation media;~~

(ii) concentrating the first complex in the presence of a second polyanion using a concentration channel in a microfluidic device, the concentration channel having at least one microscale dimension of between about 0.1 and about 500 microns;

(iii) electrophoretically separating in the presence of a third polyanion the first complex from any second complex using the filled separation channel using a separation channel in a microfluidic device, the separation channel having at least one microscale dimension of between about 0.1 and about 500 microns;

(iv) measuring an amount of the separated first complex or an amount of the separated second complex; and

(v) determining an amount of the analyte in the sample on the basis of the measured amount; wherein the first, ~~and second,~~ and third polyanions reduce interference with the determination.

43. (Previously presented) The method of claim 1, wherein the sample is selected from a serum, a plasma, a whole blood, a tissue extract, a cell extract, a nuclear extract, a culture media, a microbial culture extract, members of a molecular library, a clinical sample, a sputum specimen, a stool specimen, a cerebral spinal fluid, a urine sample, a uro-genital swab, a throat swab, or an environmental sample.

44. (Previously presented) The method of claim 1, wherein the analyte is one or more selected from alpha feto protein (AFP), human chorionic gonadotropin (hCG), thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), interleukin, Fas ligand, cancer antigen 19-9 (CA19-9), cancer antigen 125 (CA125), prostate specific antigen (PSA), hepatitis B virus antigen (HBsAg), anti-HIV antibody, or thyroxine (T4).

45-52 (Canceled)

53. (Currently amended) The method of claim [[51]] 1, wherein contacting the sample containing the analyte with one or more conjugates to form a complex of the analyte and the conjugate is conducted in a microchannel fluidically connected to the concentration channel having at least one microscale dimension of between about 0.1 and 500 microns.

54. (Canceled)

55. (Currently amended) The method of claim [[51]] 1, wherein concentrating the complex is conducted by utilizing the difference in an electrophoretic mobility between the complex and noise constituents in the sample on the basis of charge of the charged carrier molecule.

56. (Currently amended) The method of claim [[51]] 1, wherein concentrating the complex is conducted by utilizing the difference in an adsorption property between the complex and noise constituents in the sample on the basis of charge of the charged carrier molecule.

57. (Currently amended) The method of claim [[51]] 1, wherein concentrating the complex is conducted according to a concentration method selected from field amplification sample stacking (FASS), field amplification sample injection (FASI), isotachopheresis (ITP), isoelectric focusing (IF), and [[or]] solid phase extraction (SPE).

58. (Currently amended) The method of claim [[51]] 1, wherein concentrating the complex is conducted according to a concentration method selected from field amplification sample stacking (FASS), and [[or]] isotachopheresis (ITP).

59-65. (Canceled)

66. (Currently amended) The method of claim [[62]] 1, wherein the one or more synthetic sequences comprises one or more nucleotides selected from a phosphorothioate analog of nucleotide, a nucleotide that contains a methylene group in the place of the oxygen in the ribose ring, or a nucleotide in which a replacement for the 2'-sugar deoxy substituent is selected from a 2'-fluoro, 2'-O-methyl, 2-O-alkoxyl, and 2'-O-allyl modification

67-85. (Canceled)

86. (Currently amended) The method of claim [[51]] 1, wherein the concentration media comprises a size exclusion resin, a polyacrylamide gel, polyethylene glycol (PEG), polyethyleneoxide (PEO), a co-polymer of sucrose and epichlorohydrin, polyvinylpyrrolidone (PVP), hydroxyethylcellulose (HEC), poly-N,N-dimethylacrylamide (PDMA), or an agarose gel.

87. (Canceled)

88. (Currently amended) The method of claim [[51]] 1, wherein the second polyanion is added to the concentration media at a concentration of between about 0.01 to 5%.

89. (Currently amended) The method of claim [[51]] 1, wherein the second polyanion is added to the concentration media at a concentration of between about 0.05 to 2%.

90. (Canceled)

91. (Currently amended) The method of claim [[51]] 1, wherein the ~~second~~ first polyanion comprises heparin sulfate which is ~~added to the sample buffer~~ present at a concentration of between about 0.001 to 2%.

92. (Original) The method of claim [[51]] 1, wherein the concentration channel has at least one cross-sectional microscale dimension of between about 0.1 and 200 microns.

93-94. (Canceled)

95. (Previously presented) The method of claim 1 or 37, wherein two or more conjugates are used, and wherein each affinity molecule in the two or more conjugates has a property capable of binding with the analyte at a different site on the analyte from every other affinity molecule.

96. (Previously presented) The method of claim 10 or 38, wherein each conjugated and non-conjugated affinity molecule has a property capable of binding with the analyte at a different site on the analyte from every other affinity molecule.

97. (Canceled)

98. (Previously presented) The method of claim 39, wherein two or more conjugates are used, and wherein each affinity molecule in the two or more conjugates has a property capable of binding with the analyte in the sample and the labeled analyte at a different site on the analyte in the sample and a different site on the labeled analyte from every other affinity molecule, or each affinity molecule in the conjugate has a property capable of binding with the analyte in the sample and the labeled analogue at a different site on the analyte in the sample and a different site on the labeled analogue from every other affinity molecule.

99. (Previously presented) The method of claim 41, wherein two or more affinity molecules are used, and wherein each affinity molecule has a property capable of binding with the analyte in the sample and the labeled analyte at a different site on the analyte in the sample and a different site on the labeled analyte from every other affinity molecule, or each affinity molecule has a property capable of binding with the analyte in the sample and the labeled analogue at a different site on the analyte in the sample and a different site on the labeled analogue from every other affinity molecule.

100. (Previously presented) The method of claim 42, wherein two or more affinity molecules are used, and wherein each affinity molecule has a property capable of binding with the analyte in the sample and the charged carrier molecule-bound analyte at a different site on the analyte in the sample and a different site on the charged carrier molecule-bound analyte from every other affinity molecule, or each affinity molecule has a property capable of binding with the analyte in the sample and the charged carrier molecule-bound analogue at a different site on the analyte in the sample and a different site on the charged carrier molecule-bound analogue from every other affinity molecule.

101. (Previously presented) The method of claim 1, wherein the step (i) further comprises:

adding the first polyanion to a solution containing the sample containing the analyte; and
contacting the solution and the one or more conjugates to form a complex of the analyte and the one or more conjugates in the presence of the first polyanion.

102. (Previously presented) The method of claim 1, wherein the step (i) further comprises:

adding the first polyanion to a solution containing the one or more conjugates; and

contacting the solution and the sample containing the analyte to form a complex of the analyte and the one or more conjugates in the presence of the first polyanion.

103. (Previously presented) The method of claim 10, wherein the step (i) further comprises:

adding the first polyanion to a solution containing the sample containing the analyte; and
contacting the solution and the one or more conjugates and the one or more non-conjugated affinity molecules to form a complex of the analyte, the at least one conjugate, and the at least one non-conjugated affinity molecule in the presence of the first polyanion.

104. (Previously presented) The method of claim 10, wherein the step (i) further comprises:

adding the first polyanion to a solution containing the one or more conjugates and/or the one or more non-conjugated affinity molecules; and

contacting the solution and the sample containing the analyte and, if not present in the solution, the one or more conjugates or the one or more non-conjugated affinity molecules, to form a complex of the analyte, the at least one conjugate, and the at least one non-conjugated affinity molecule in the presence of the first polyanion.

105. (Previously presented) The method of claim 37, wherein the step (i) further comprises:

adding the first polyanion to a solution containing the sample containing the analyte; and
contacting the solution and the one or more conjugates, wherein at least one of the one or more conjugates is labeled by a detectable marker, to form a complex containing the analyte and the at least one conjugate in the presence of the first polyanion.

106. (Previously presented) The method of claim 37, wherein the step (i) further comprises:

adding the first polyanion to a solution containing the one or more conjugates, wherein at least one of the one or more conjugates is labeled by a detectable marker; and

contacting the solution and the sample containing the analyte to form a complex containing the analyte and the at least one conjugate in the presence of the first polyanion.

107. (Previously presented) The method of claim 38, wherein the step (i) further comprises:

adding the first polyanion to a solution containing the sample containing the analyte; and
contacting the solution and the one or more conjugates and the one or more non-conjugated affinity molecules, wherein either at least one of the conjugates or at least one of the non-conjugated affinity molecules is labeled by a detectable marker, to form a complex containing the analyte, the at least one conjugate, and the at least one non-conjugated affinity molecule in the presence of the first polyanion.

108. (Previously presented) The method of claim 38, wherein the step (i) further comprises:

adding the first polyanion to a solution containing the one or more conjugates and/or the one or more non-conjugated affinity molecules; and

contacting the solution and the sample containing the analyte and, if not present in the solution, with the one or more conjugates or the one or more non-conjugated affinity molecules, wherein either at least one of the conjugates or at least one of the non-conjugated affinity molecules is labeled by a detectable marker, to form a complex containing the analyte, the conjugate, and the non-conjugated affinity molecule in the presence of the first polyanion.

109. (Previously presented) The method of claim 39, wherein the step (i) further comprises:

adding the first polyanion to a solution containing the sample containing the analyte; and
contacting the solution, either the labeled analyte or the labeled analogue, and the one or more conjugates to form the first complex and the second complex in the presence of the first polyanion.

110. (Previously presented) The method of claim 39, wherein the step (i) further comprises:

adding the first polyanion to a solution containing the one or more conjugates; and
contacting the solution, the sample containing the analyte, and either the labeled analyte or the labeled analogue to form the first complex and the second complex in the presence of the first polyanion.

111. (Previously presented) The method of claim 41, wherein the step (i) further comprises:

adding the first polyanion to a solution containing the sample containing the analyte; and
contacting the solution, either the labeled analyte or the labeled analogue, the one or more conjugates, and the one or more non-conjugated affinity molecules to form the first complex and the second complex in the presence of the first polyanion.

112. (Previously presented) The method of claim 41, wherein the step (i) further comprises:

adding the first polyanion to a solution containing the one or more conjugates and/or the one or more non-conjugated affinity molecules; and

contacting the solution, the sample containing the analyte, either the labeled analyte or the labeled analogue, and, if not present in the solution, the one or more conjugates or the one or more non-conjugated affinity molecules, to form the first complex and the second complex in the presence of the first polyanion.

113. (Previously presented) The method of claim 42, wherein the step (i) further comprises:

adding the first polyanion to a solution containing the sample containing the analyte; and
contacting the solution, either the charged carrier molecule-bound analyte or the charged carrier molecule-bound analogue, and the labeled affinity molecule to form the first complex and the second complex in the presence of the first polyanion.

114. (Previously presented) The method of claim 42, wherein the step (i) further comprises:

adding the first polyanion to a solution containing the labeled affinity molecule; and
contacting the solution, the sample containing the analyte, and either the charged carrier molecule-bound analyte or the charged carrier molecule-bound analogue to form the first complex and the second complex in the presence of the first polyanion.

115-120. (Canceled)